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INAUGURAL DR. GIULIO J. D'ANGIO AWARD

In 1940, the diagnosis of Wilms' tumor was associated with the same poor prognosis as that of other forms of childhood cancer. The addition of radiation therapy, and then chemotherapy to the management plan for these children improved their prognosis. However, the more aggressive therapy caused late complications which were of concern to the pioneers of pediatric oncology.

Giulio J. D'Angio, M.D., has focused his professional career on recognizing the need for an appreciation of the balance that must be achieved between successful therapy and reduction of late morbidity from treatment. The National Wilms' Tumor Study Group (NWTSG), which he founded, has completed four studies which had as their goals increasing the survival rate of children with Wilms' tumor and improving the quality of life of the successfully treated children. No one has been more thoughtful or energetic than Dr. D'Angio in the pursuit of these goals. The results of the NWTSG therapeutic trials have formed the basis for the treatment of children with Wilms' tumor on all continents, treatment which will lead to a population of adults who have been both cured of their tumor and spared much of the morbidity, thought, prior to the first National Wilms' Tumor Study in 1969, to be an unavoidable cost of successful treatment.

Throughout his career, Dr. D'Angio has fostered the development of young investigators, recognizing both the importance of these for the continued evolution of pediatric oncology, and their "endangered species" status in the present rapidly changing fiscal and academic environment. The organizing committee desired to honor the energy and contributions of Dr. D'Angio by establishing an award in his name to reward those we knew he would most like to reward, the young investigators who will be the future leaders in pediatric oncology.

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Cloning of Candidate Genes Involved in the Beckwith-Wiedemann Syndrome and Childhood Tumors

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INTRODUCTION

The Beckwith-Wiedemann syndrome (BWS) is characterized by several growth abnormalities, such as macroglossia, umbilical hernia and gigantism [1]. Children suffering from this syndrome have an increased risk of developing different kinds of embryonic tumors, among which Wilms' tumor is the most common [2]. The gene for familial BWS has been assigned to chromosome 11p15 by linkage studies [3,4]. This region also shows loss of heterozygosity (LOH) in BWS-associated tumors. In some BWS patients, chromosomal aberrations on 11p15 have been found. These are duplications, which are always of paternal origin, translocations, which are always of maternal origin, and paternal uniparental disomy. These findings and the fact that in familial cases the inheritance is predominantly maternal suggest that genomic imprinting is involved. In addition, in the BWS-associated tumors, the LOH found on 11p15 is always maternal which also points to genomic imprinting [5].

We analyzed eight balanced translocation breakpoints on 11p15 using fluorescence in situ hybridization (FISH) and pulsed field gradient electrophoresis (PFGE). These breakpoints were mapped to three chromosomal regions.

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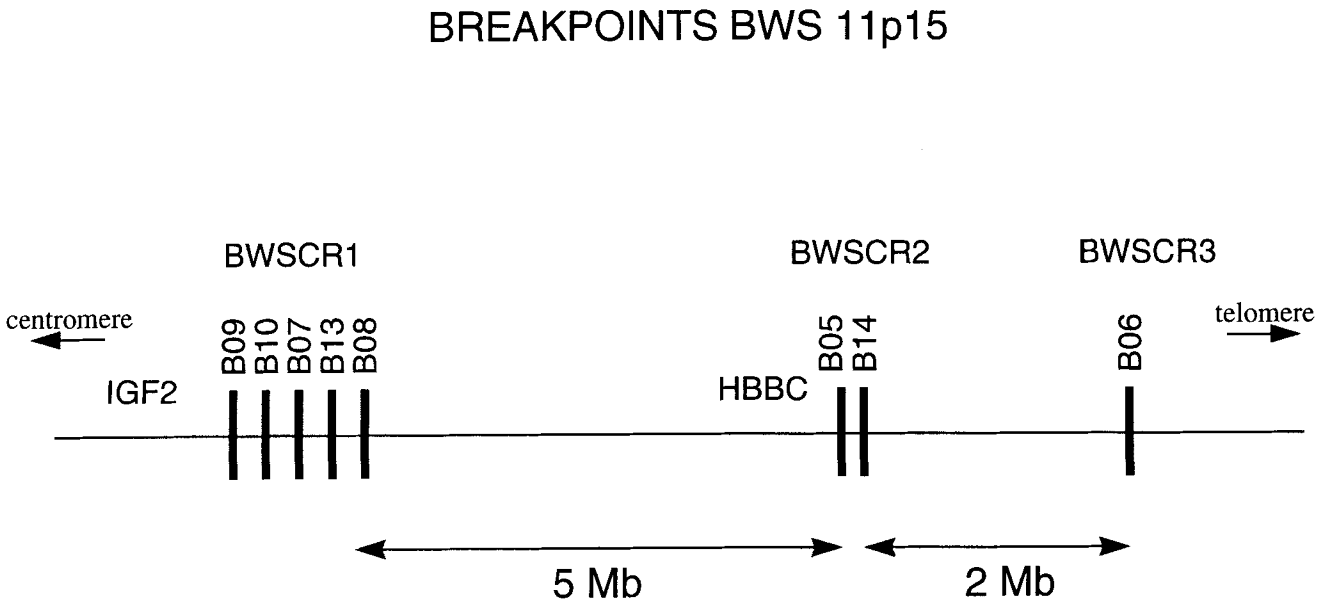

Fig. 1. An overview of the Beckwith-Wiedemann syndrome chromosome regions on 11p15. Five breakpoints are clustered near IGF2. Five Mb more proximal, another two breakpoints are located. One breakpoint is located an additional 2 Mb proximal.

TABLE I. Clinical Features of the Patients

	B05	B06	B07	B08	B09	B10	B13	B14
Increased birth weight					+	+	+	+
Macroglossia		+	+	+	+	+	+	
Ear lobe grooves	+	+	+	+			+	
Umbilical hernia		+	+	+	+	+	+	
Visceromegaly		+						
Inguinal hernia		+	+					
Hypoglycemia		+	+	+				+
Hemihypertrophy	+							+
Tumor	+							

Five clustered proximal to Insulin-Like Growth Factor 2 (IGF2) (Beckwith-Wiedemann syndrome chromosomal region 1[BWSCR1]), another two proximal to the hemoglobin beta gene cluster (HBBC) (BWSCR2), and the last breakpoint mapped about 2 Mb even more proximal (BWSCR3, Fig. 1). Considering the clinical features of the patients, they can be divided into two groups. The two patients associated with BWSCR2 both have minor signs of BWS, show hemihypertrophy and one developed a Wilms' tumor. All other patients have all classical signs of BWS (Table I).

In order to clone genes involved in BWS, yeast artificial chromosome (YAC) and cosmid contigs were constructed overlapping breakpoints in BWSCR1 and 2. BWSCR1 consists of five breakpoints and spans 460 kb. In this region, CpG islands were cloned. At least two of the clones are evolutionary conserved and detect a 6.5-kb transcript in all tissues tested (see manuscript M. Mannens in this issue). BWSCR2 consists of two breakpoints, separated by less than 25 kb. A YAC spanning these breakpoints was subcloned in cosmids and a 150-kb cosmid contig was constructed. Hybridization of these cosmids to zinc-finger linker-specific oligonucleotides and subsequent sequencing showed that zinc-finger sequences were present on both sides of the breakpoints. The direction of transcription of the zinc-finger regions is opposite, meaning that they belong to two different genes. Hybridization of Krüppel-associated box (KRAB)-specific oligonucleotides revealed the presence of one KRAB domain, which was confirmed after sequencing (Fig. 2). Almost one-third of all zinc-finger genes contain a KRAB domain. This domain has been shown to repress transcription in transcription experiments [6,7]. These are good candidate genes for BWS since the overgrowth symptoms and the increased tumor risk point to a deregulation of growth control. Because no transcripts were detected on Northern blots and cDNA library screening did not produce positive clones, we used reverse transcriptase polymerase chain reaction (RT-PCR) to isolate transcribed sequences from lymphocyte RNA. Using poly dT, a primer from the zinc-finger region and a primer from the KRAB domain, we were able to isolate a 2,3-kb spliced PCR product containing four zinc-fingers and a KRAB domain.

In summary, the chromosomal breakpoints on 11p15 of BWS patients with balanced translocations are divided into three regions. The clinical heterogeneity among the patients correlates with different breakpoint regions. The two patients associated with BWSCR2 both have minor signs of BWS and have hemihypertrophy, while patients

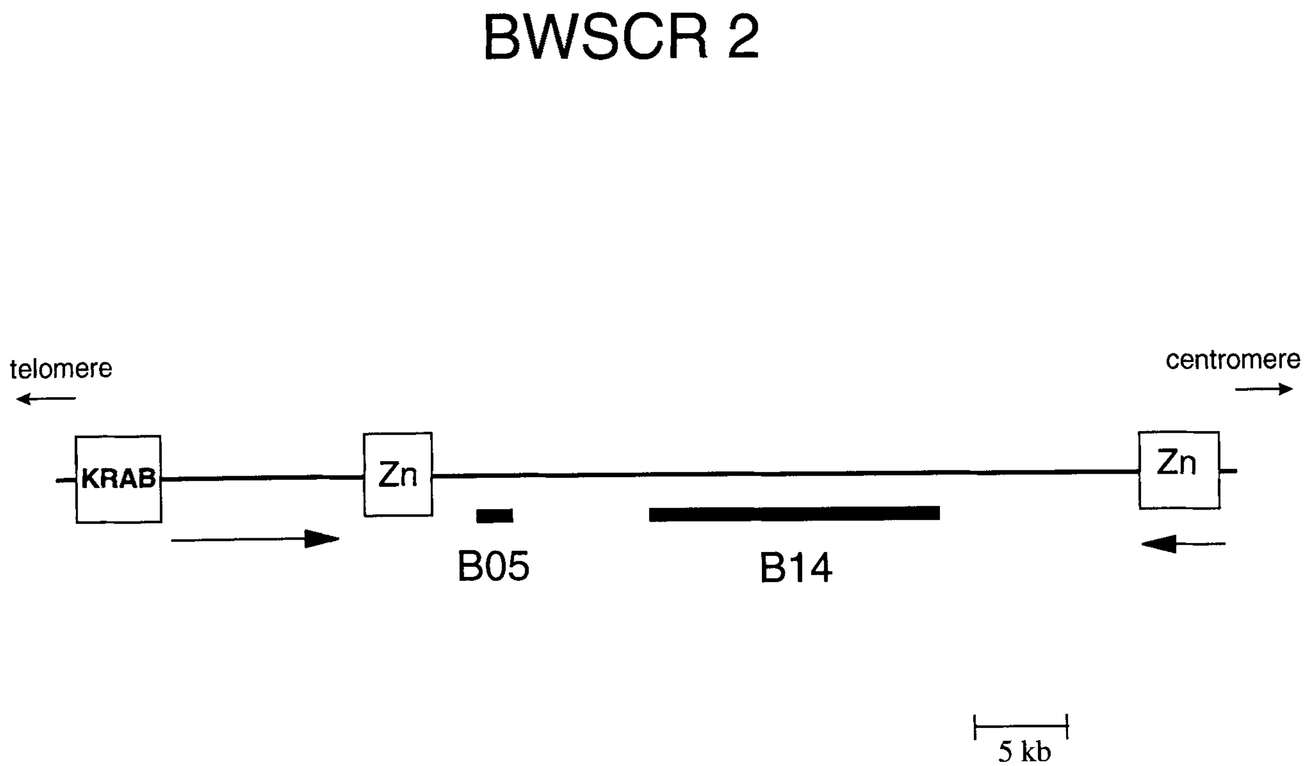

Fig. 2. A schematic representation of BWSCR2. The position of the zinc-finger regions and the KRAB domain are indicated. The arrows indicate the direction of transcription. The bars indicate the position of the breakpoints.

in BWSCR1 and 3 are all clear BWS patients. In the most distal BWS region, we found conserved CpG islands which detect a 6.5-kb transcript and we were able to clone a zinc-finger gene near BWSCR2. These data suggest the involvement of multiple genes in the etiology of BWS.

REFERENCES

1. Pettenatti MJ, Haines JL, Higgins RR, Wappner RS, Palmer CG, Weaver DD: Wiedemann-Beckwith syndrome: Presentation of clinical and cytogenetical data on 22 new cases and review of the literature. Hum Genet 74:143–154, 1986.
2. Wiedemann H: Tumours and hemihypertrophy associated with Wiedemann-Beckwith syndrome. Eur J Pediatr 141:129, 1983.
3. Ping AJ, Reeve AE, Law DJ, Young MR, Boehnke M, Feinberg AP: Genetic linkage of BWS to 11p15. Am J Hum Genet 44:720–723, 1989.
4. Koufos A, Grundy P, Morgan K, Aleck KA, Hadro T, Lampkin BC, Kalbakji A, Cavenee WK: Familial Wiedemann-Beckwith syndrome and a second Wilms' tumor locus both map to 11p15.5. Am J Hum Genet 44:711–719, 1989.
5. Mannens M, Hoovers JMN, Redeker E, Verjaal M, Feinberg AP, Little P, Boavida M, Coad N, Steenman M, Bliek J, Niikawa N, Tonoki H, Nakamura Y, Boer de EG, Slater RM, John R, Cowell JK, Junien C, Henry I, Tommerup N, Weksberg R, Pueschel SM, Leschot NJ, Westerveld A: Parental imprinting of human chromosome region 11p15.3-pter involved in the Beckwith-Wiedemann syndrome and various human neoplasia. Eur J Hum Genet 2:3–23, 1994.
6. Margolin JF, Friedman JR, Meyer WKH, Vissing H, Thiesen HJ, Rauscher FJ III: Krüppel-associated boxes are potent transcriptional repression domains. Proc Natl Acad Sci USA 91:4509–4513, 1994.
7. Witzgall RR, O'Leary E, Leaf A, Önaldi D, Bonventre JV: The krüppel-associated box-A (KRAB-A) domain of zinc finger proteins mediates transcriptional repression. Proc Natl Acad Sci USA 91:4514–4518, 1994.